CHAPTER 6. THE ECOLOGY AND DIVERSITY OF MACROFUNGI FRUITING ON SOIL WITH SPECIAL REFERENCE TO THE ECTOMYCORRHIZAL SPECIES

Introduction

Macrofungi fruiting on soil are comprised of ectomycorrhizal species and decomposer species. This chapter examines the contribution to the study of these two highly important groups of macrofungi, which may be different or overlapping in function, e.g. there is evidence that ectomycorrhizal fungi produce extracellular enzymes and are able to metabolise soil carbon by acting as decomposers (Talbot *et al*. 2008). The relationship between the regenerating forests at different ages after wildfire disturbance and the ensuing ectomycorrhizal community is of interest for the concept of sustainable forest management in the wet *E. obliqua* forests of Tasmania and is given special attention.

The function of the ectomycorrhizal and decomposer species on soil

The fruit bodies of the ectomycorrhizal species are the visible above-ground manifestation of the symbiotic function of mycorrhizality, one of the most significant processes in a terrestrial ecosystem. The pioneering observations of Reissek (1847) (*fide* Last *et al*. 1987) and Frank (1885) established the mycorrhizal symbiosis between a fungus and the host tree. This symbiosis is a state of mutualistic association whereby the plant host and mycorrhizal fungus co-exist in a physiologically, ecologically and reproductively active state for long periods of time (Harley 1989). Mycorrhizae increase the absorptive capacity of the root system of the plant host and help it to survive against temperature extremes and in low nutrient soils as well as protecting it against disease; in return, the non-photosynthetic fungus receives translocated organic carbon as photosynthates (Smith and Read 2008). In addition, the same species of ectomycorrhizal fungus present on more than one host may enable transfer of carbon across source-sink gradients, which would have implications for productivity during drought or where nutrients are limiting (Simard *et al.* 1997).

Ectomycorrhizal fungi can form distinct homogenous masses of densely interwoven rhizomorphs, strands or hyphae belonging to the same species of fungus called 'ectomycorrhizal mats' (Unestam 1991). These mats can affect the chemical composition of the associated soils and aid in litter decomposition by creating air pockets which induce arthropod activity, leading to faster lignin and cellulose decomposition (Cromack Jr. *et al*. 1988, Entry *et al*. 1991, Unestam 1991). Furthermore, in Douglas-fir conifer forests these mats have been shown to support seedlings by transferring energy and possibly water and nutrients from the overstorey trees to the shade-intolerant seedlings (Griffiths *et al*. 1991).

Macrofungal fruit bodies of both ectomycorrhizal and decomposer species are able to concentrate nutrients at differing levels (Vogt *et al*. 1981, Lindeberg 1981), yet they have structural and functional similarities which are suspected to lead to conflict and competition for these nutrients, especially nitrogen and phosphorus in the soil (Leake *et al*. 2002). Ectomycorrhizal fungi were found to suppress the activity of saprotrophic organisms in the litter of a *Pinus radiata* (gymnospermic) plantation (Gadgil and Gadgil 1971), although this was not the case in mature beech (angiospermic) forests in a similar study by Staaf (1988).

In this study, some of the macrofungal species that are not currently classified as ectomycorrhizal (and so are classified as decomposers) may have some type of symbiotic function not yet identified, or, be partially mycorrhizal with the symbiosis being of short duration, e.g. *Entoloma* spp. (Kobayashi and Hatano 2001). Those species classified as decomposers that do grow on the soil rather than directly out of litter may also be involved in breaking down the last remnants of the litter that have become part of the humic layer (Hering 1982).

Ecological studies of macrofungi fruiting on soil

Phenological studies of macrofungi fruiting on soil date back to 1788 (Grainger 1946). Lange (1978) wrote that only a modest number of studies have been devoted to the phenology and ecology of ectomycorrhizal fungi but, by 1953 at least 70 papers dealing with the ecology of higher fungi, including fungi fruiting on soil, had been reviewed (Hering 1966). The majority of these ecological studies have been undertaken in the Northern Hemisphere, especially in European countries which have

a long cultural history of mycophagy. It was observed that different species of soil-inhabiting macrofungi grew under different tree species (Maire *et al*. 1901), on different soil types (Haas 1933) and were associated with different plant communities (Wilkins *et al*. 1937). Lange (1978) observed that a time of up to two weeks and even longer was needed for the fruiting bodies of large species to appear following rain. He also noted that half the total number of species found fruiting on soil were known to be ectomycorrhizal. Bohus and Babos (1960) found that the differences in the pH values of the soil did not always parallel the differences in forest types and their associated mycota. Holownia (1985) concluded that the seasonal phenology in the appearance of fruit bodies of fungi belonging to species common for two areas under study was not the same in each of the two communities in spite of the short distance (700m) separating them and that this difference was no doubt due to different edaphic conditions. Tyler (1985) concluded that decomposer species were characterised by the less acid soils and the ectomycorrhizal species by the more acid soils.

Changes in macrofungal communities on soil have been used to reflect the effects of pollution and reduction of habitat due to agriculture and urban development in The Netherlands (Arnolds 1988) and in Sweden (Rühling and Tyler 1990). Contrived field experiments such as the addition of nitrogen to a reforested 35 year old spruce stand (Peter *et al*. 2001b) resulted in the above-ground ectomycorrhizal fungi reducing dramatically in numbers after one year of nitrogen addition. The below-ground community was less affected until after 2 years. Similarly, significant changes in ectomycorrhizal species richness were found over an anthropogenic nitrogen gradient, with only 14 species producing sporocarps at high mineral nitrogen concentrations compared with 144 species at low nitrogen concentrations (Lilleskov *et al*. 2001). Observations from 10 years data that annual fruit body production is extending later into the year suggest the effects of climate change (Watling 2004).

Ectomycorrhizal succession with stand age

The response of fungal communities to forest succession is largely unknown (Smith *et al*. 2002). The concept of early and late stage fungi (Dighton and Mason 1985, Last *et al*. 1987) pertains to a sequence in which species of *Hebeloma, Laccaria* and *Inocybe* are characterised as early stage, *Cortinarius* and *Tricholoma* as intermediate stage and *Russula, Amanita* and *Leccinum* as late stage symbionts. Dighton and

Mason (1985) suggest that this succession is meant to be a result of the tree ageing and the ensuing alteration of the resources available to the mycorrhizas and their host plants. However, no apparent differences in the occurrence of mycorrhizal types in relation to tree age were found in a glasshouse study on two *Picea abies* stands of 1 year old seedlings and 8-10 year old trees (Blasius and Oberwinkler 1989).

Smith *et al*. (2002) examined the species diversity and abundance of epigeous and hypogeous ectomycorrhizal fungi in stands of Douglas-fir (*Pseudotsuga menziesii*) of three different ages, viz. young (30-35 years), rotation-age (45-50) and old growth (>400 years) and found that fruit body production was significantly greater in young and rotation-age stands compared with old growth stands. It was also found that species or species groups were unique to an age class but that the most dominant genera appeared in all age classes. However, the claim by Smith *et al*. (2002), that 25% of the genera found appeared exclusively in either young or old growth stands, supports the genus-level patterns of ectomycorrhizal succession as forests age as proposed by Dighton and Mason (1985). Even so, many of the genera characterised as early or late stage in other studies, e.g. Fox (1986), were multi-aged in the study of Smith *et al*. study. Visser (1995) found that the number of ectomycorrhizal fungi was much higher in jack pine stands of 41, 65 and 122 years after wildfire than after 6 years, the complexity of species composition increasing with time since wildfire until stabilisation at 41 years. That study also found discrepancies in the early and late stage fungi concept, e.g. the absence of *Laccaria* spp. in the 6 year old stand. Such discrepancies among studies imply that it is difficult to generalise patterns of ectomycorrhizal succession between different forest types and to define ecological traits common to all species (Smith *et al*. 2002). The concept of ‘early and late stage’ was criticised by Newton (1992) who suggested that classification of ectomycorrhizal fungi based on the epidemiological characteristics which determine competitive ability would be a more functional classification. Although emerging thought now is that in native forest ecosystems it is an oversimplification to use the terms ‘early stage’ and ‘late stage’ (Twieg *et al*. 2007), the terms are still useful for describing ectomycorrhizal community structure in the current study.

Studies on ectomycorrhizal fungi in Australia

The studies of Samuel (1926), Chilvers and Pryor (1965), Chilvers (1968a, 1968b) Ashton (1976a) and McGee (1986) were concerned with discovering which plants in Australia formed mycorrhizal associations and the structure and function of mycorrhizae, as it was recognised that ectomycorrhizal fungi were essential to plant health and development. Little was known about the ectomycorrhizal diversity and ecology of these fungi in Australian forests until 1988, when a project was initiated to collect, isolate and identify ectomycorrhizal fungi from forests throughout Australia (Castellano and Bougher 1994). That study revealed that Australia contains an extraordinary high number of ectomycorrhizal hypogean fungi. Harley and Smith (1983) suggested that ectomycorrhizae occur predominantly in climates of periodic drought. It is speculated that the underground fruit body is an adaptation to the arid conditions experienced over much of Australia (Claridge 2002). Hypogean fungi have an ecological impact on the ecosystem beyond those of improving soil quality and maintaining plant health. These fungi are a key food source of the diet of many small mammals especially after fire (Taylor 1992, Johnson 1995, Claridge 2002). This relationship means that the fungi are effectively dispersed over long distances, as the spores remain viable after passing through the intestinal tract of the animal.

About 660 species of ectomycorrhizal fungi have been named in Australia (Bougher 1995). However, given that perhaps only 5-10% of Australian fungi have been named and another 10% are known but not named the true number of ectomycorrhizal fungi is more in the vicinity of 6500 species (Bougher and Syme 1998). Papers of an ecological nature have been the result of a growing awareness that an understanding of fungal biology and ecology is essential in mycorrhizal management in forestry (Castellano and Bougher 1994). This is due to the role of ectomycorrhizal fungi in the maintenance of plant diversity in natural ecosystems and those disturbed by management. Castellano and Bougher (1994) emphasise the continued need for taxonomic information as the basis for assessing this role. Tommerup and Bougher (2000) label ectomycorrhizal fungi as ‘a critical ecosystem resource’. Their point of view is that an undisturbed mature forest contains the baseline data of diversity for ecosystem functioning. This view is not universally held, as it is considered that a mature forest is only one stage of a forest ecosystem subject to natural disturbances

that set benchmarks for all stages along the successional pathway (T. Wardlaw, pers. comm. 2009).

Ectomycorrhizal fungal diversity was used in two studies, Gardner and Malajczuk (1988) and Glen *et al*. (2008) to assess the recolonisation of rehabilitated bauxite mine sites in Western Australia. Counts of fruit bodies and morphotypes of ectomycorrhizae were used by Gardner and Malajczuk (1988) to compare diversity between rehabilitated sites and native forest. The study of Glen *et al*. employed fruit body counts and molecular sequencing of root tips. Both studies showed an increase in the ectomycorrhizal species richness with time since rehabilitation although the study of Glen *et al*. found a higher richness in the 7 year old stands since rehabilitation than that of Gardner and Malajczuk, which may have been due to the longer length of time of the Glen *et al*. study or to the use of advanced molecular techniques in that study or both. Newbound (2009) examined the effects of urbanization on the diversity of epigeous ectomycorrhizal macrofungi in remnant eucalypt woodlands in Victoria and found that the ectomycorrhizal community appeared not to be affected by urbanization.

A report in Tasmania (Marsden-Smedley 1989) did not find any relationship between ectomycorrhizal fungi and different forest types. This could have been due to the limited survey time of that study and the inexperience of the surveyor. Gates *et al*. (2009) examined the effect of aggregated retention silviculture on the ectomycorrhizal community in a wet *E. obliqua* forest and found that the aggregates retained a substantial population of species of ectomycorrhizal fungi relative to the native forest control, although the species compositions were not identical.

Other information pertaining to the diversity and distribution of Australian ectomycorrhizal fungi tends to be that gathered as part of inventories in different forest types before or after disturbances, e.g. Packham *et al*. (2002), Syme (2004), Ratkowsky and Gates (2005), Robinson and Tunsell (2007) and Catcheside and Catcheside (2005, 2008). In such studies, the life mode of the fungus may or may not be recorded (although it can usually be inferred from the fungal species) and the species host tree/shrub may or may not be noted. No further statistical analyses were provided separately for the ectomycorrhizal species recorded but perhaps these

otherwise very informative studies could be analysed at a later stage for more information.

The potential of ectomycorrhizal fungi to influence plant diversity and productivity is now recognised and, reciprocally, so is the role of the plant community in determining the ectomycorrhizal fungal communities (van der Heijden *et al*. 1998). Natural distributions of ectomycorrhizal fungi can provide insights about their potential responses to anthropogenic disturbances. Klironomos and Kendrick (1993) reported that 700 publications were generated yearly pertaining to mycorrhiza, the majority of which were concerned with vascular arbuscular mycorrhizae and increased plant production, the latter being of societal interest. However, despite the recognition of the importance of ectomycorrhizal fungi to ecosystem functioning and the establishment of ectomycorrhizal associations for the health of a forest ecosystem as a prime consideration in evaluating the effects of disturbances, both natural and anthropogenic, field ecological studies of these organisms are few (Klironomos and Kendrick 1993, Erland and Taylor 2002). Ectomycorrhizal fungal species richness and community structure may be threatened by humans gathering for commercial gain in native forests and by the loss of habitat due to anthropogenic activities (e.g. clearing of forests for agriculture and urban development and intensive forestry) (Arnolds 1991, Watling 2005). Other disturbances contributing to ectomycorrhizal community structure changes are wildfire, pollution (e.g. acid rain atmospheric nitrogen deposition, fertilization) and climate change.

The aims of this chapter are:

- to establish baseline data regarding the soil-inhabiting ectomycorrhizal and decomposer macrofungal species of a native wet sclerophyll, *E. obliqua* dominated forest ecosystem in southern Tasmania subjected to wildfire disturbance.
- to investigate the influence of the vascular plant community of a regenerating *E. obliqua* forest at different ages since wildfire on the ectomycorrhizal population and attempt to identify species that may be indicators of forest age.

The questions are:

- Are there any differences in the species richness and species assemblages of the macrofungi fruiting on soil (decomposers and ectomycorrhizal species combined) among the four plots?
- Are there any differences in the ectomycorrhizal communities among the four plots?
- Is there an association between the ectomycorrhizal communities and the vascular plant community in each plot?
- Is there any evidence of ectomycorrhizal succession as reflected in the type of ectomycorrhizal species with increasing stand age?
- What are the effects of rainfall and temperature on fruit body production of the soil-inhabiting (both decomposer and ectomycorrhizal) macrofungi?
- Are there seasonal differences among the macrofungal species assemblages on soil among the four plots?

Materials and methods

Survey and laboratory methods were as described in Chapter 4. Trappe (1962), Warcup (1980), Bougher (1995) and Bougher and Syme (1998) provided knowledge on the ectomycorrhizal status of the macrofungal species. Only basidiomycetous species were included in the ectomycorrhizal analyses as the mycorrhizal status of the four species of above-ground macrofungal Ascomycetes that were found was unknown. Although it was previously believed that most Ascomycota were decomposers, recent work using morphotyping and molecular research has revealed a high diversity of ascomycetous ectomycorrhizality, especially in the Pezizales (e.g. Tedersoo *et al*. 2006). Because most of those ascomycetous taxa remain unidentified to species level and are of uncertain life mode, the classification of a macrofungal species fruiting on soil was confined to three categories, viz. Ascomycota, basidiomycetous decomposers, and basidiomycetous ectomycorrhizal. Hypogean fungi were collected if the fruit bodies appeared at the soil surface.

Macrofungal species richness was measured by the number of species present, as in Chapter 4. Species richness was analysed using randomised species accumulation curves and frequency bar graphs. To establish the relationship between species

numbers and sampling intensity, the Mao-Tau estimator in EstimateS (Colwell 2005) was used to generate randomised species accumulation curves for all macrofungi fruiting on soil for each of the four plots and all plots combined using 30 visits and 25 subplots, respectively, as the basis for replication. Randomised species accumulation curves were also used with increasing number of visits to each plot and with increasing number of subplots, i.e. increasing area for each plot, to test the effect of increased sampling intensity on ectomycorrhizal macrofungal species richness. For the latter test, the 25 subplots, each measuring 10x10m, was used as the basis for replication. For each plot separately, by choosing fixed numbers of subplots, the species numbers at those 'cut points' were expressed as a percentage of the total number of species in the plot.

Macrofungal species assemblages, including the effects of seasonality, were analysed using CAP-CDA, as in Chapter 4. The ordination procedure CAP-CDA was used to visualise and quantify differences in macrofungal species assemblages fruiting on soil among the four plots using the total number of visits in which fungi were found fruiting on soil (i.e. 114 visits). Regression analysis was used to examine the relationship between the total number of ectomycorrhizal species on soil and the total number of living vascular plant species in each of the 100 subplots. The mean numbers of ectomycorrhizal macrofungal species fruiting on soil were tabulated with the number of the three main host tree species known to form ectomycorrhizal associations (*E. obliqua*, *N. cunninghamii* and *P. apetala*) present in each subplot. To test for a correlation with the vascular plant community, macrofungal species assemblages were analysed using CAP-CCorA (Ratkowsky and Gates 2008). The 'Statistical methods' section of Chapter 4 should be consulted for a discussion of the difference between the 'species by visits' and 'species by subplots' data structures. The number of living vascular plant species (21) was examined for its correlation with the species list for each subplot of each plot. The plotting symbols chosen to display the results of CAP-CCorA represent three vegetation types, viz. 'Rainforest' (rainforest species), 'Pomaderris' and 'Monotoca' (in this study, synonymous with the 1934 plot), derived from the predominant numbers of vascular plants present in the 100 subplots.

Results

The results for the macrofungi fruiting on soil are presented in terms of species richness and species assemblages. Special attention is paid to the relationship of the ectomycorrhizal species with the vascular plant community of the subplots within each plot. Tables and figures whose names contain the letter 'A' are in Appendix 1.

Species richness of macrofungi on soil

Species identification and number of records

In total, 495 macrofungal species were recorded fruiting on soil. Of these, 330 were known to be ectomycorrhizal and 165 were considered decomposers (Appendix 2). There were 111 ectomycorrhizal species found only once during the 14 months survey period, 57 that occurred twice and 32 species that occurred three times. The 1898/1934 plot had the highest number of ectomycorrhizal species (179) and the 1898 plot had the least (92) (Figure 6.1 and Table 6.A1). The highest number of decomposers (96) was in the Old growth plot and the lowest (49) was in the 1934 plot. The number of soil-inhabiting species of the Ascomycota was very low (7) and they were distributed evenly across the plots. The highest number of soil-inhabiting basidiomycetous macrofungal species was in the 1898/1934 plot (260), followed by the Old growth plot (202). The highest number of records of soil-inhabiting macrofungi was in the 1898/1934 plot (1781), followed by the number in Old growth (1647); by comparison, the 1898 and 1934 plots had approximately 500 records less. The genus with the highest number of species was *Cortinarius* (231 species, including *Dermocybe*, *Cuphocybe* and *Rozites*) of which only 14 could be identified to species level. *Lactarius eucalypti* was the most often recorded ectomycorrhizal basidiomycetous macrofungus (347 records). *Laccaria* spp. (a composite species group) was second with 332 records, *Cortinarius* 'C248 varnished with umbo' 121 records, *Russula persanguinea* 111 records, *Hydnum repandum* 81 records, *Cortinarius rotundisporus* 80 records, *Descolea recedens* 70 records and *D. phlebophora* 69 records.

Figure 6.1 depicts the numbers of species and life mode on soil for each of the four plots and all plots combined. The ectomycorrhizal species was relatively higher than the number of decomposers in the two younger plots (1934 and 1898/1934).

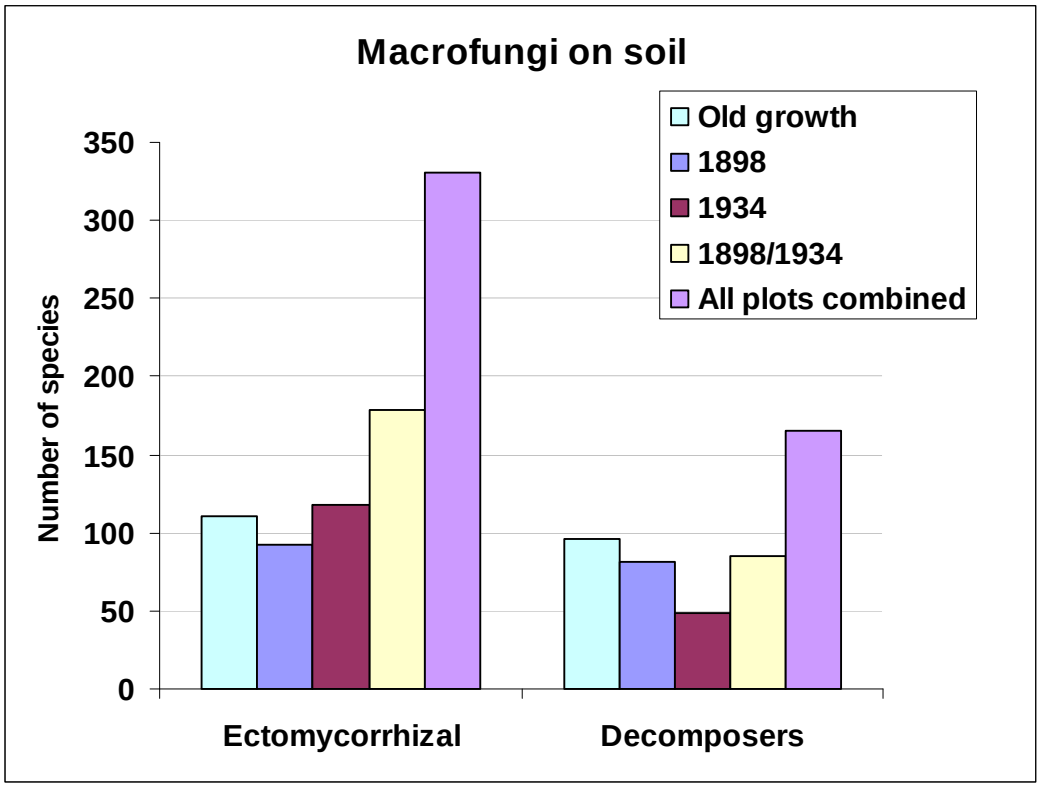


Fig. 6.1. Species numbers of the ectomycorrhizal and decomposer macrofungal species found on soil in the four plots and all plots combined.

The distribution of the major ectomycorrhizal families across the four plots and all plots combined is shown in Figure 6.2. It can be seen quite clearly that the family Cortinariaceae dominates over the other families in all four plots with the highest number of species from this family being in the 1898/1934 plot.

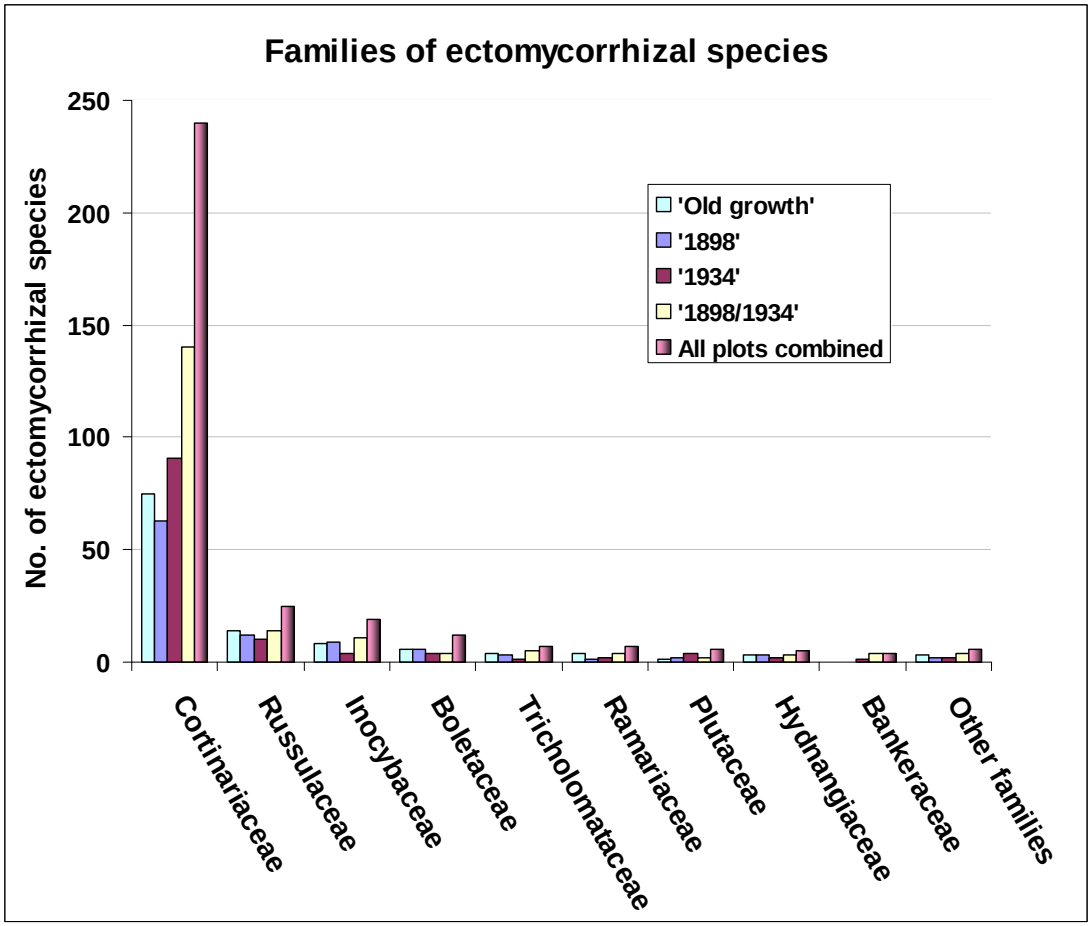
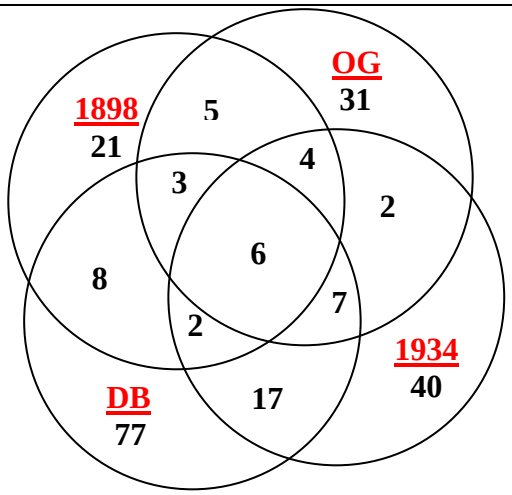


Fig. 6.2. The distribution of species in families of ectomycorrhizal fungi fruiting on soil across the four plots and all plots combined.

The distribution of 231 *Cortinarius* species found in the four plots is summarised in a Venn diagram in Figure 6.3. It can be seen that the two younger plots, 1934 and 1898/1934 (DB), shared 17 species in common that were found only in these two plots. The mature plots 1898 and Old growth had 5 species in common that were only found in these two plots. There were 6 species common to all four plots, viz. *Cortinarius rotundisporus*, *C. submagellanicus*, *C.* 'C110 sandy ochre with white downy covering', *C.* 'C200 ochre-brown with clear umbo, spores 8x4', *C.* 'C248 varnished with umbo' and *Dermocybe clelandii*. The 1898/1934 plot had the highest number of unique species (77) followed by 1934 (40), then Old growth (31) and 1898 (21).



Intersections not shown:

DB and OG:	**5**
1898 and 1934:	**3**

Fig. 6.3. Venn diagram showing the distribution of the 231 *Cortinarius* species in the four plots (Note: DB = 1898/1934).

Randomised species accumulation curves

In Figure 6.4, the curves for the 1898 and 1934 plots are almost superimposed on each other, meaning that new species were found at approximately the same rate in these two plots. The curve for the 1898/1934 plot is distanced from these two curves, which implies that species accumulated at a faster rate in that plot. The curve for the Old growth plot is intermediate between this one and the other two. It does not appear that an asymptote is being approached by any of the four curves.

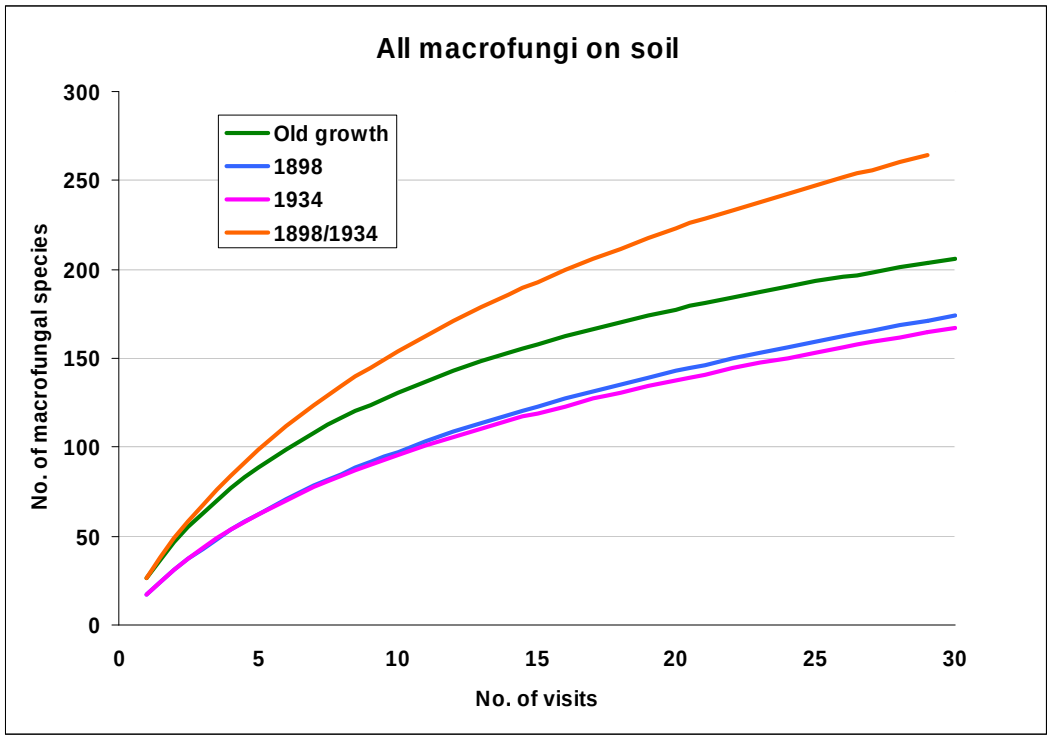


Fig. 6.4. Randomised species accumulation curves for each of the four plots for all macrofungi fruiting on soil, based on visits.

In the following graph (Figure 6.5) using the 25 subplots, the curves are ever increasing, although at a reduced rate, with no suggestion of an asymptote being approached, which is similar to Figure 6.4.

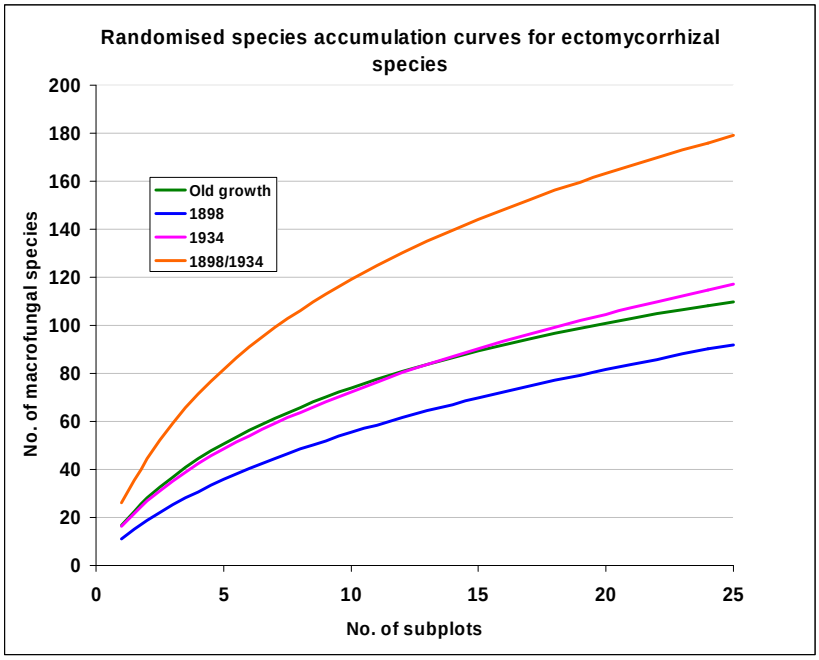


Fig. 6.5. Randomised species accumulation curves for the number of ectomycorrhizal macrofungi fruiting on soil in each plot with increasing area (number of subplots).

In Table 6.1, it can be seen that ca. 46% of the number of ectomycorrhizal species found for the Old growth and 1898/1934 plots were collected at 5 subplots, which have a total area of 500m^2. At 15 subplots (1500m^2), more than 80% of the total number of ectomycorrhizal species found had been collected for Old growth and 1898/1934 and more than 75% for 1898 and 1934.

Table 6.1. Species numbers of ectomycorrhizal fungi at 5, 10, 15, 20 and 25 subplots expressed as a percentage of the total number of ectomycorrhizal species for each plot using Mao-Tau estimates, after 30 visits.

Plot\subplots	5(=500m^2)	10(=1000m^2)	15(=1500m^2)	20(=2000m^2)	25(=2500m^2)
OG	46%	67.2%	81.3%	91.8%	100%
1898	38.9%	60.2%	75.8%	88.7%	100%
1934	41.4%	61.9%	77.1%	89.5%	100%
1898/1934	45.6%	66.6%	80.5%	91.2%	100%

Effects of rainfall and temperature

In Figure 6.6. although rainfall was high in July 2006 – October 2006 and maximum temperatures were still low relative to the hotter months (i.e. November – February), the number of macrofungal species fruiting on soil decreased dramatically over this

period. Very good rains in April 2006 (265.8mm) and May 2006 (162.8mm) preceded the appearance of fruit bodies. The number of species found peaked in May 2007 and June 2007 in the Old growth and 1898/1934 plots. Many more species were found in the 1898/1934 plot in May – June 2007 than in May – June 2006. From September 2006 – February 2007, more species were found in the Old growth plot than in any other plot in a period of increasing temperatures and sporadic rainfall events.

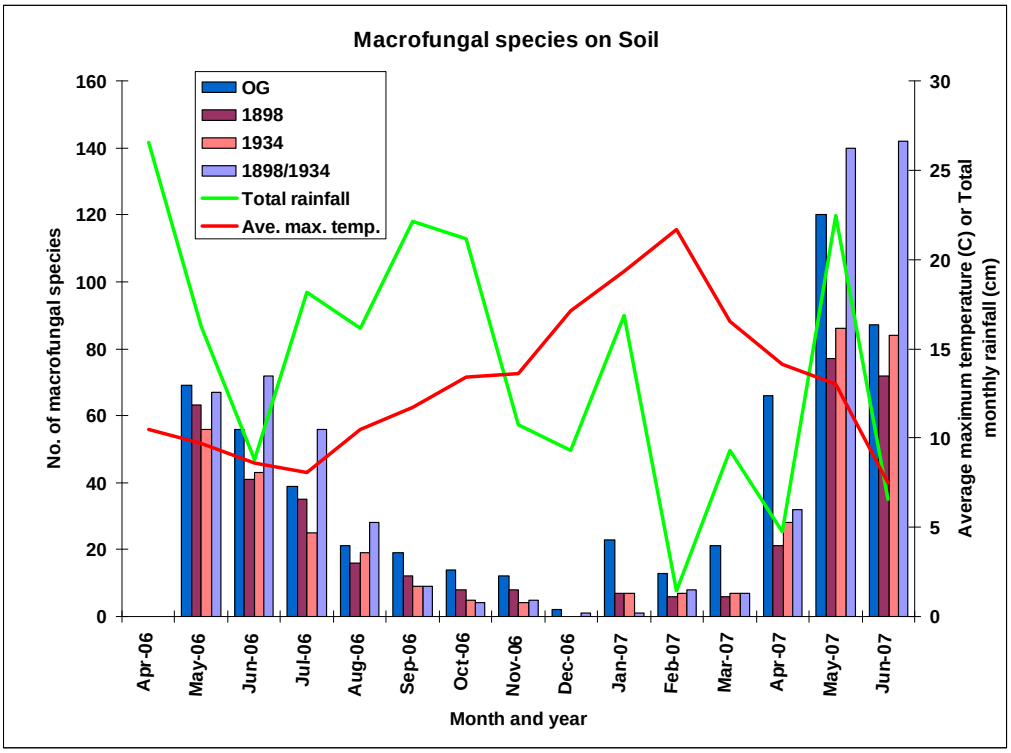


Fig. 6.6. The effects of rainfall and temperature on macrofungal species on soil.

Species assemblages of macrofungi on soil

Assemblage composition

The resulting ordination diagrams for CAP-CDA are shown in Figure 6.7. This constrained ordination of all macrofungi fruiting on soil in the four plots using visits as replication shows that each plot has a distinct mycota (with some degree of overlap) (P-value of 0.00001 from 99,999 permutations, misclassification rate 14%). This reflects the relative stability of the macrofungal assemblages within each plot over time.

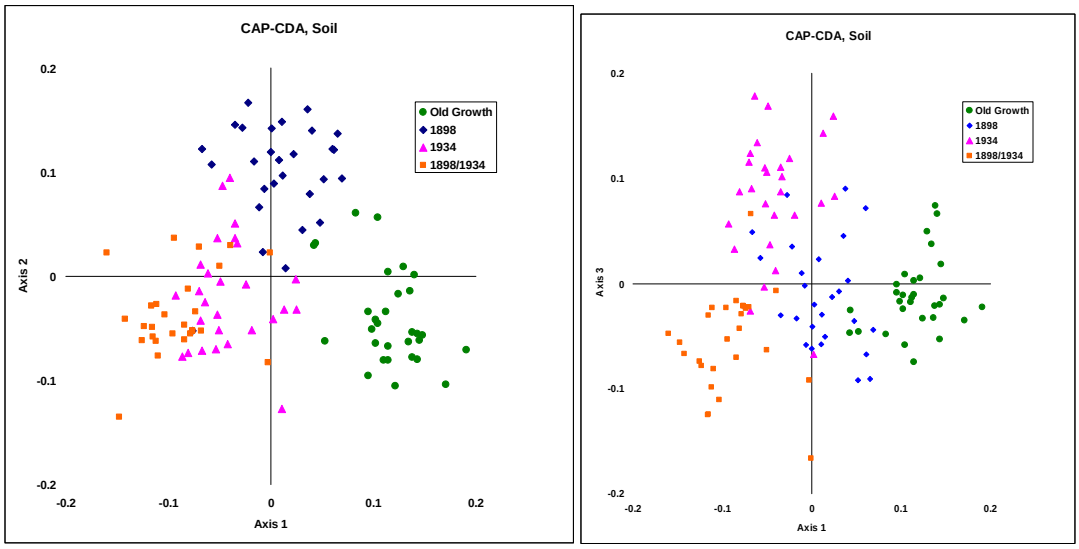


Fig. 6.7. CAP-CDA for all macrofungi fruiting on soil (495 species), using visits as replication.

The CAP-CDA procedure was also done for all macrofungi fruiting on soil using subplots as replication. The ordination diagrams in Figure 6.8 show that the points within each of the four individual plots are clumped tightly, reflecting the fact that differences among the mycota of the 25 subplots are less than among plots. The misclassification rate, using the 'leave one out' procedure, was 2%, much lower than the 14% misclassification rate obtained using visits as replication (Figure 6.7), suggesting that the mycota is more stable spatially than temporally.

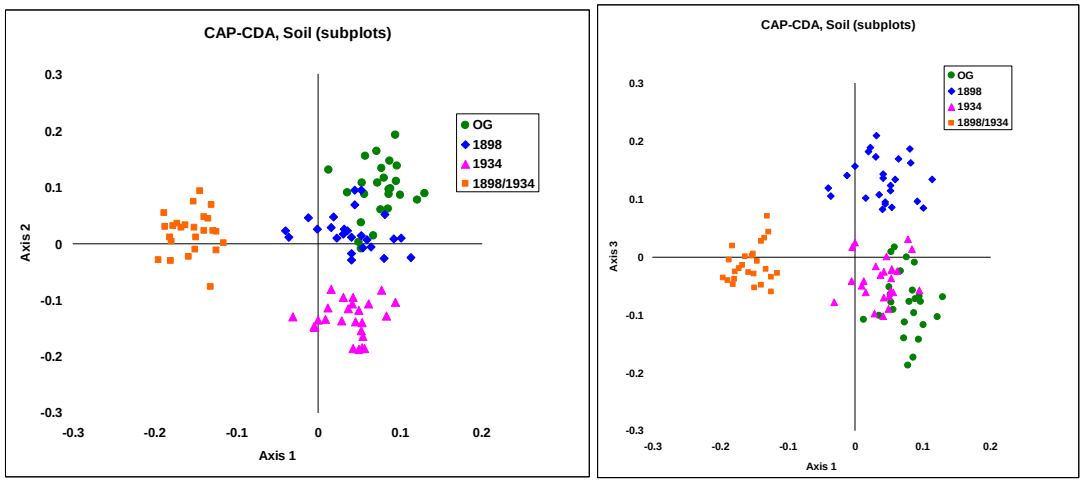


Fig. 6.8. CAP-CDA for the total number of macrofungi fruiting on soil (495 species) using the 100 subplots as replication.

The procedures MDS (Figure 6.A1), PCOA (Figure 6.A2), and CAP-CDA (Figure 6.9) were carried out using 114 visits for those species on soil that have an ectomycorrhizal life mode. Similar to the results for all mycota on soil, the four plots group distinctly (misclassification rate 14.3%, P-value of 0.00001 from 99,999 permutations), reflecting the stability of the ectomycorrhizal community of each plot over time. The MDS and PCOA ordinations (Figure 6.A1 and Figure 6.A2) also indicate plot differences but not as convincingly as CAP-CDA.

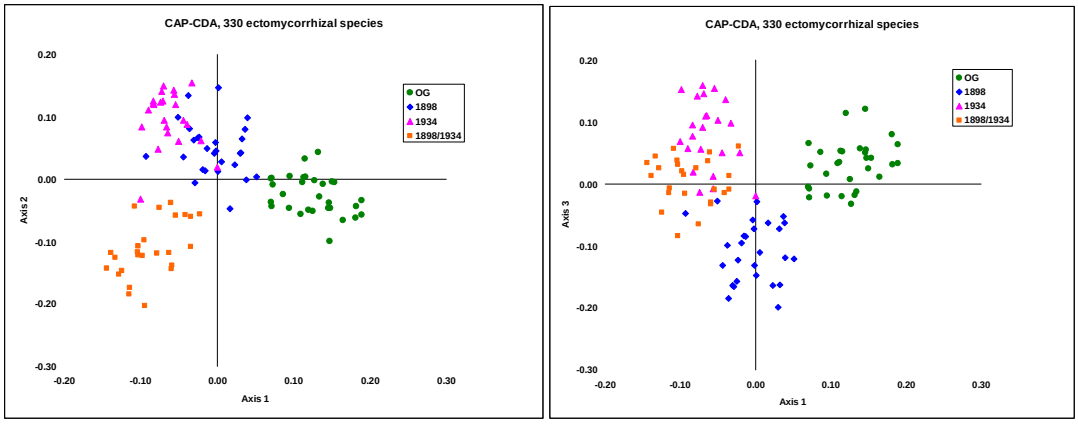


Fig. 6.9. CAP-CDA on ectomycorrhizal species fruiting on soil, using visits as replication.

PCOA (Figure 6.A3) and CAP-CDA (Figure 6.10) were also carried out for those species on soil that have an ectomycorrhizal life mode using the 25 subplots of each plot rather than visits as replication. The groupings for the constrained ordination are even clearer than in Figure 6.9, where visits were used as replication, again reflecting a greater stability in space than over time. The misclassification rate from CAP-CDA, using the 'leave one out' procedure, was 3.0%. The permutation test gave a P-value of 0.00001 from 99,999 permutations.

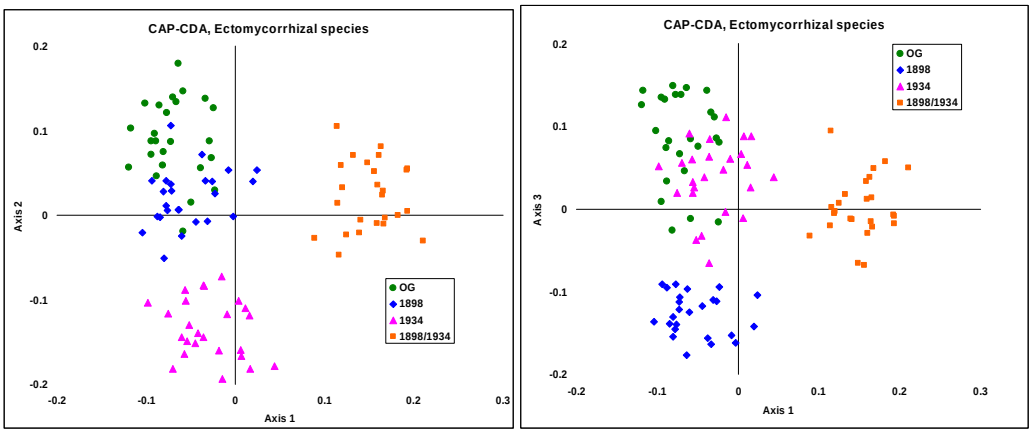
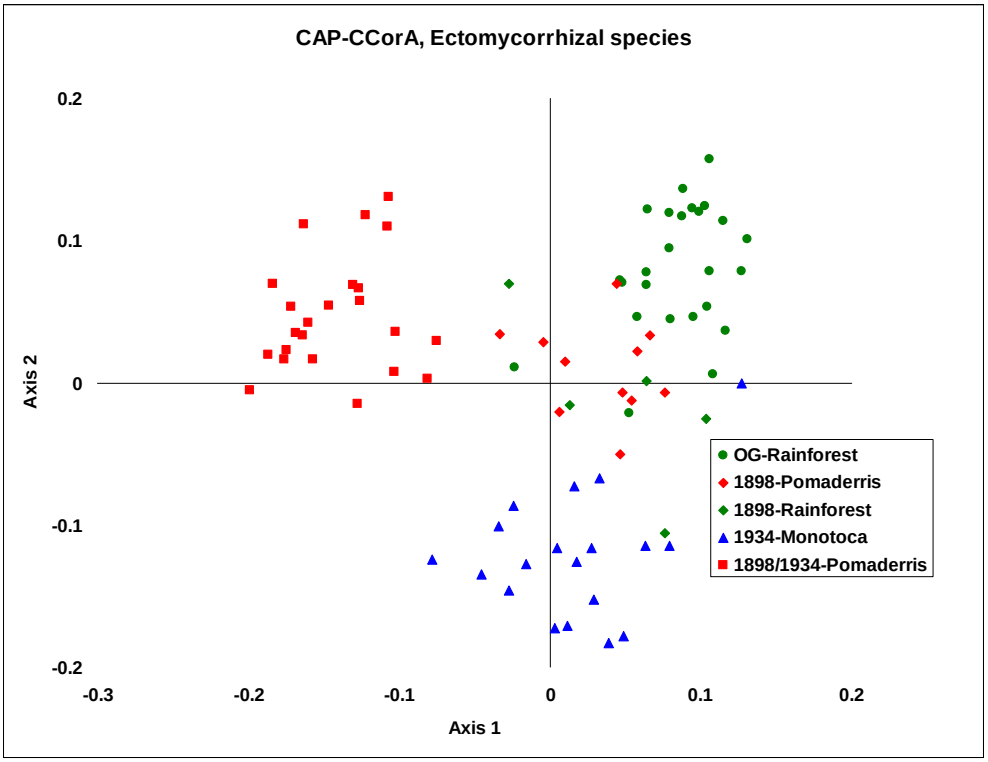


Fig. 6.10. CAP-CDA on the ectomycorrhizal species fruiting on soil, using subplots as replication.

Ectomycorrhizal species assemblages correlated with vascular plant species

A highly significant correlation using CAP-CCorA (Figure 6.11) was obtained (P-value of 0.00001 from 99,999 permutations), indicating that the ectomycorrhizal species are correlated with the chosen vegetation types. The 'Monotoca' subplots form a tight group. The majority of the points from the '1898/1934–Pomaderris' combination (red squares) group tightly but the subplots of the '1898–Pomaderris' combination (red diamonds) overlap with 'Rainforest'. The OG–'Rainforest' combination overlaps with the '1898–Rainforest' combination.



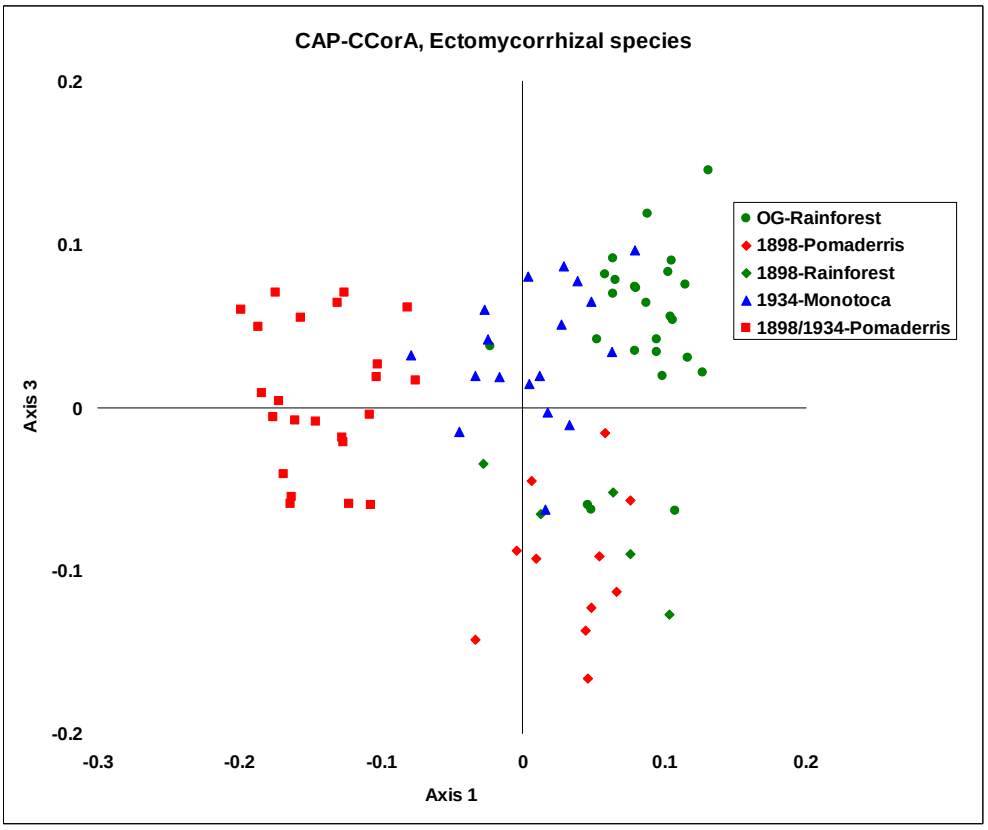


Fig. 6.11. CAP-CCorA between the vascular plants present in each of 100 subplots and the lists of ectomycorrhizal species fruiting on soil obtained from repeated visits to those subplots.

In Figure 6.12, which correlates the total number of ectomycorrhizal species on soil with the total number of living vascular plant species in each of the 100 subplots, the points on the graph have considerable scatter. However, the correlation between macrofungal species numbers and tree numbers is significant (P<0.0001). Subplots having more than 50 living trees all have at least 22 ectomycorrhizal species, whereas subplots having less than 50 living trees have an average of ca. 15 ectomycorrhizal species.

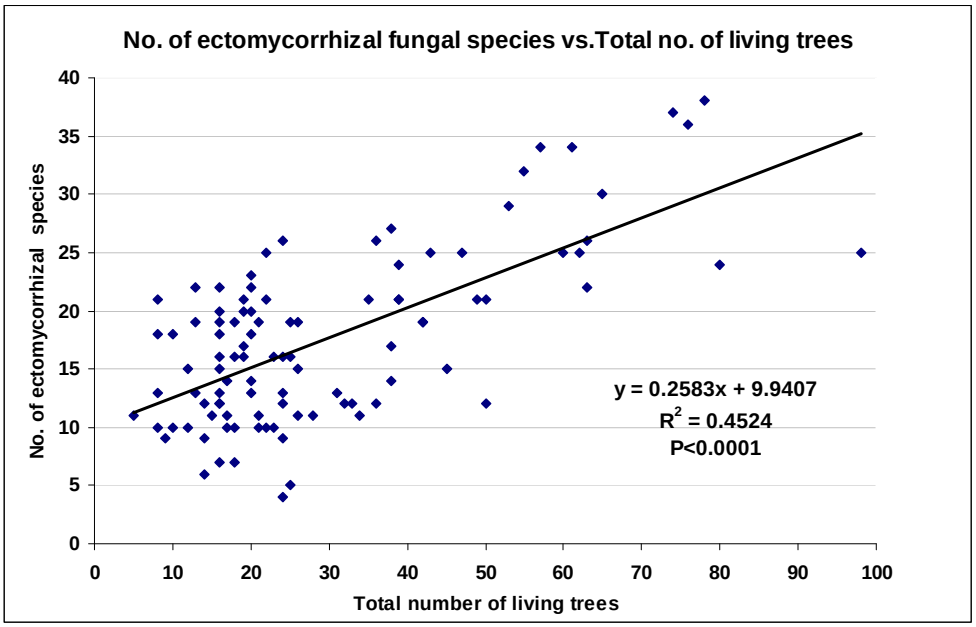


Fig. 6.12. Ectomycorrhizal macrofungal species on soil correlated with the total number of living trees in each of the 100 subplots.

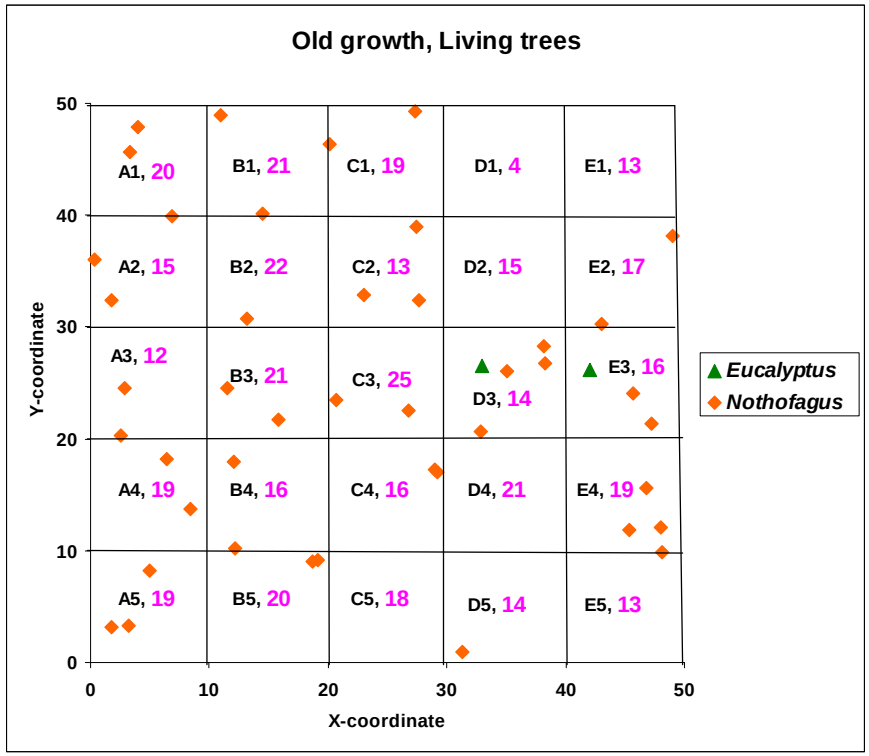
Increasing the number of host species (Table 6.2) does not appear to affect the number of ectomycorrhizal species of fungi found. Regression analyses of the number of ectomycorrhizal host stems against number of ectomycorrhizal macrofungal species and against surface area of CWD also produced non-significant correlations.

Table 6.2. Mean numbers of ectomycorrhizal fungal species with the three main ectomycorrhizal host species (*E. obliqua*, *N. cunninghamii* and *P. apetala*) in the subplots of each plot.

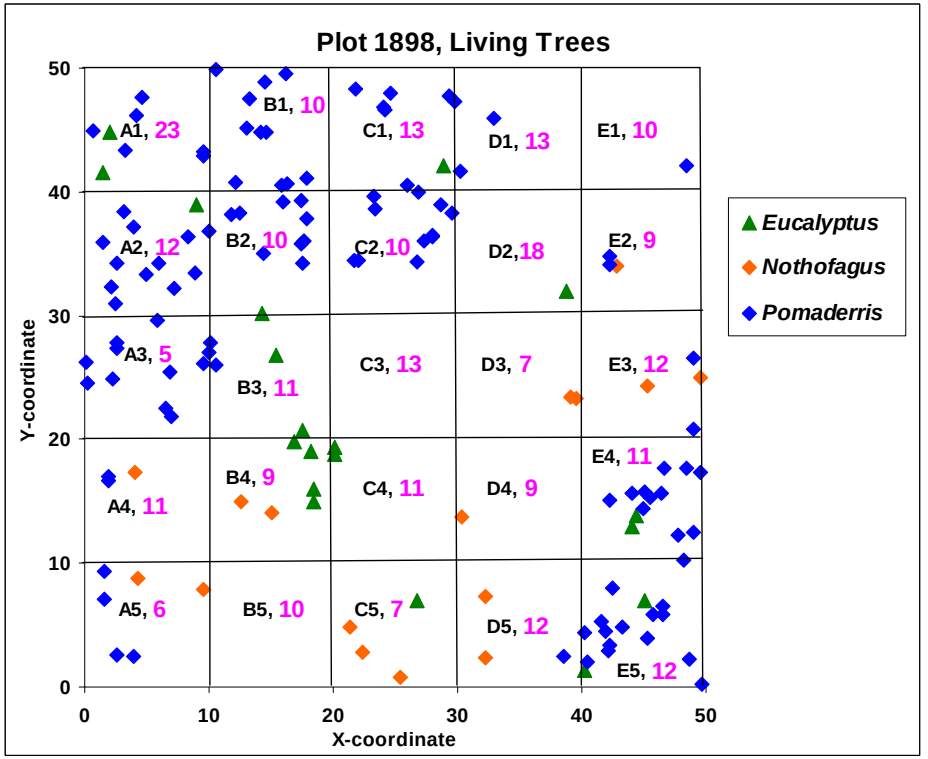
Plot	Number of ectomycorrhizal hosts		
	1 species only	2 species	All 3 species
OG	16.9	15.0	Not applicable
1898	10.3	11.3	11.2
1934	14.2	17.2	Not applicable
1898/1934	Not applicable	24.7	26.6

Distribution of ectomycorrhizal species within a plot

The number of ectomycorrhizal species within a subplot is shown in the following maps (Figure 6.13(a-d)), together with the positions of the three main living ectomycorrhizal host tree species with stems ≥10cm in diameter, *viz*. *Eucalyptus obliqua*, *Nothofagus cunninghamii*, *Pomaderris apetala*. The distribution of ectomycorrhizal species numbers does not appear to correlate with the locations of the living ectomycorrhizal hosts. For example, in 1898 (Figure 6.13b), there is a lack of trees across the plot diagonal from lower left to upper right, yet there are still quite high numbers of ectomycorrhizal fungi in that region.



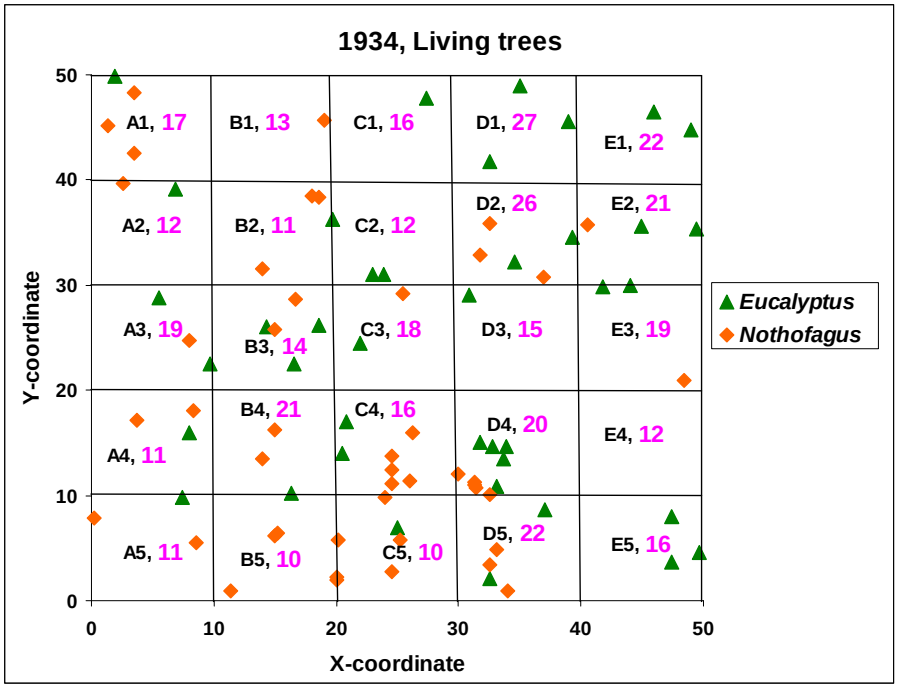
a)



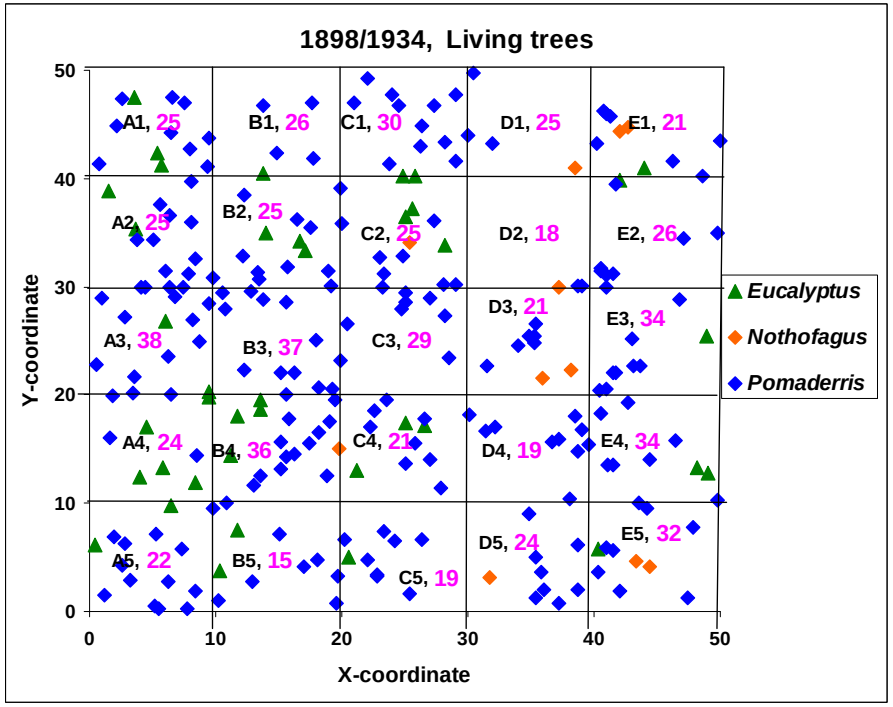
b)

Fig. 6.13(a-b). The position of the three main living ectomycorrhizal host tree species with the numbers of ectomycorrhizal macrofungal species fruiting on soil in each subplot of each plot.

(continued next page)



c)



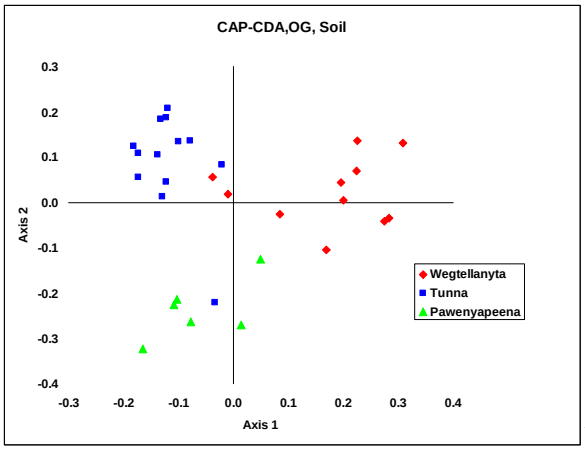
d)

Fig. 6.13(c-d). The position of the three main living ectomycorrhizal host tree species with the numbers of ectomycorrhizal macrofungal species fruiting on soil in each subplot of each plot.

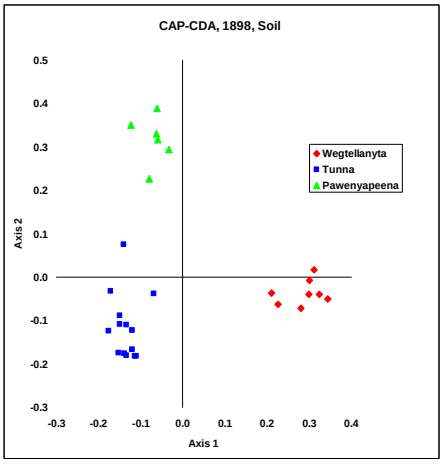
Seasonality for macrofungi on soil

The effect of using the indigenous seasons (see Chapter 3, Statistical methods) to explain macrofungi fruiting on soil was investigated using PCOA (Figure 6.A4) and CAP-CDA procedures. The ordination diagrams of the CAP-CDA for indigenous seasons are given in Figure 6.14 for each of the plots separately, since previous results (e.g. Figures 6.7 and 6.8) indicated that there was a strong plot effect present. The P-values and misclassification rates for both indigenous and traditional seasons are given in Tables 6.3 and 6.4, respectively. Seasonal differences in macrofungal species assemblage composition are better described using indigenous seasons rather than traditional seasons. Table 6.3 shows that the Old growth plot had the highest number of visits misclassified and 1934 the least. The P-values and misclassification rates are compared to the P-values and misclassification rates using traditional seasons in Table 6.4, which reveals that even though the P-values using traditional seasons are significant, the misclassification rates are very high in comparison to the misclassification rates for indigenous seasons.

a)



b)



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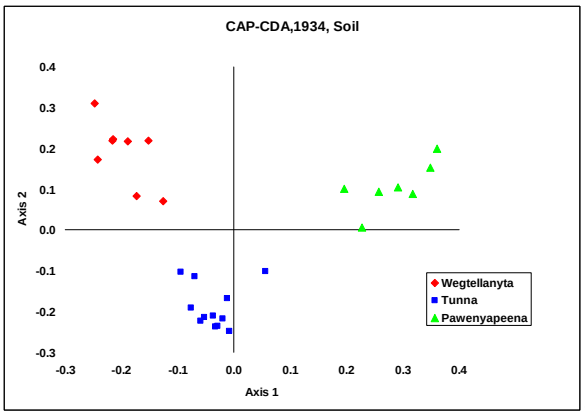


d)

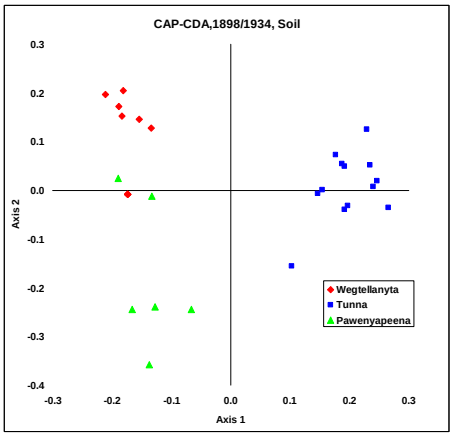


Fig. 6.14(a-d). CAP-CDA, macrofungi fruiting on soil, for each of the four plots using the three indigenous seasons as the predefined groups (Wegtellanyta: Dec.-Apr.; Tunna: May-Aug.; Pawenyapeena: Sept.-Nov.).

Table 6.3. P-values and misclassification rates from 99,999 permutations for all macrofungi on soil for each of the four plots using indigenous seasons.

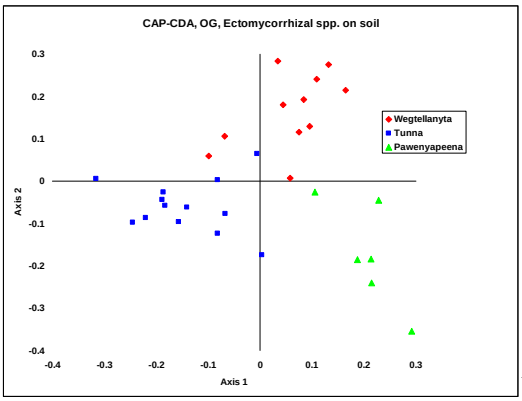
Plot	P-value, trace criterion	P-value, delta criterion	Misclassification rate
Old growth	0.00001	0.00014	17.0%
1898	0.00001	0.00008	10.7%
1934	0.00001	0.00001	7.4%
1898/1934	0.00140	0.00005	10.3%

Table 6.4. P-values and misclassification rates from 99,999 permutations for all macrofungi on soil for each of the four plots using traditional seasons.

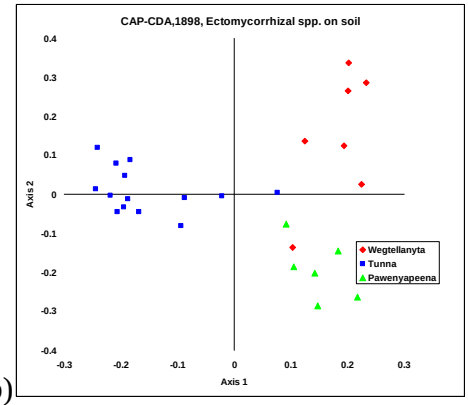
Plot	P-value, trace criterion	P-value, delta criterion	Misclassification rate
Old growth	0.00001	0.00121	23.3%
1898	0.00001	0.00599	21.4%
1934	0.00001	0.00001	29.6%
1898/1934	0.00001	0.00001	20.7%

CAP-CDA and PCOA were carried out using only the data for ectomycorrhizal species to determine the effect of indigenous seasons on that life mode. The resulting CAP-CDA diagrams are presented in Figure 6.15 and those for PCOA are shown in Figure 6.A5 (see Appendix 1). The P-values and misclassification rates for CAP-CDA are given in Table 6.5. Distinct groupings can be discerned for each plot in the ordination diagrams of Figure 6.15(a-d), indicating that seasonal differences in the ectomycorrhizal mycota are better explained by the use of indigenous rather than traditional seasons. Results for indigenous seasons are compared to those obtained with traditional seasons in Table 6.6. The misclassification rate ranges from 18.5% to 39.1% for ectomycorrhizal fungi using traditional seasons compared to a range of 8.7%-23.3% obtained using the indigenous seasons.

a)



b)



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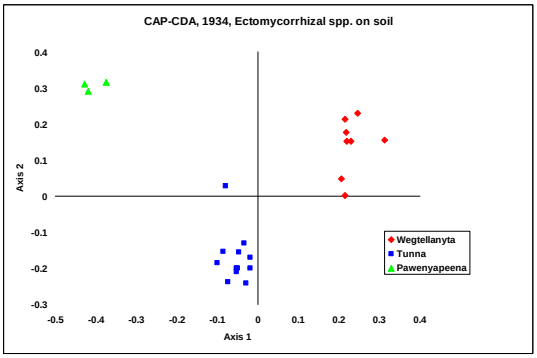

d)

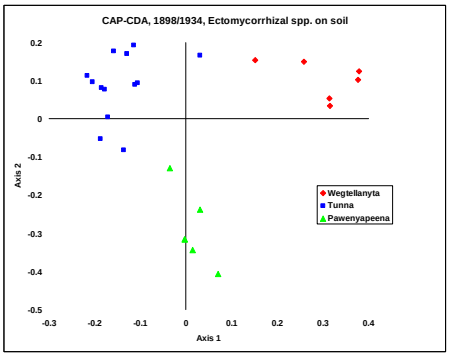

Fig. 6.15(a-d). CAP-CDA on soil ectomycorrhizal species and the effect of indigenous seasons.

Table 6.5. P-values and misclassification rates for ectomycorrhizal fungi fruiting on soil for each of the four plots using indigenous seasons from 99,999 permutations.

Plot	P-value, trace criterion	P-value, delta criterion	Misclassification rate
Old growth	0.00002	0.00445	23.3%
1898	0.00001	0.00005	14.8%
1934	0.00005	0.00161	8.7%
1898/1934	0.00001	0.00004	16.0%

Table 6.6. P-values and misclassification rate for ectomycorrhizal fungi on soil using traditional seasons from 99,999 permutations.

Plot	P-value, trace criterion	P-value, delta criterion	Misclassification rate
Old growth	0.00004	0.00178	30.0%
1898	0.00001	0.00017	18.5%
1934	0.00178	0.00365	39.1%
1898/1934	0.00002	0.00890	24.0%

Timelines for macrofungal species on soil

One of the informative aspects of a study such as this one with repeated sampling at fortnightly intervals over a period of time is that appearance and disappearance of fruit bodies can be observed. These timelines are presented in Table 6.A3 for all soil fungi that have 5 or more records. The saprotrophic genus *Agaricus* appeared in April or May and fruited until July. The majority of species of the very large ectomycorrhizal genus *Cortinarius* s.l. (i.e. including *Cuphocybe*, *Dermocybe* and *Rozites*) appeared at the beginning of May and disappeared at the end of July, with only a handful of exceptions that had longer fruiting periods. *Hebeloma* 'medium pink buff' showed the same fruiting period as the majority of the *Cortinarius* species. Members of the family Russulacaeae and the boletes generally emerged very early in the year. The saprotrophic genus *Hygrocybe* was mostly confined from April to the end of August with *H. chromolimonea*, *H. mavis* and *H.* 'vine-top with yellow gills' being exceptions. Fruit bodies of a few species appeared intermittently all year round, viz. *Descolea recedens, Discinella terrestris, Entoloma aromaticum, E. fibrosopileatum, Laccaria* spp. and *Lactarius eucalypti*.

Potential indicator species of stand age

There were many species that were specific to one of the four plots; however, as there was no replication of the plots with respect to their fire history only those species that are supported with results from other studies in Tasmania (e.g. Packham *et al*. 2002, Ratkowsky and Gates 2005) are considered. Species only found in Old growth for the course of the survey period were: *Cuphocybe* 'C162', *Geoglossum cookeanum*, *Laccaria* sp. A, *Hygrocybe rodwayi*, *Cortinarius* '*green gills*', *Plectania campylospora, Hygrocybe roseoflavida, Galerina* 'scurfy', *Laccaria masonii*, *Dermocybe* 'felty', *Dermocybe* 'greyish yellow', *Dermocybe* 'brown with orange margin', *Hygrocybe reesiae*, *Pholiota fieldiana*, *Dermocybe* 'goldy', *Camarophyllopsis* 'brown, no odour', *Camarophyllopsis* 'brown with mothball odour', *Camarophyllopsis darwinensis*', *Inocybe* 'very large, nodulose spores', *Dermoloma* 'brown-yellow', and *Dermoloma* 'grey'. *Clitocybe clitocyboides* and three species of *Agaricus* were found only in 1898, *viz*. *Agaricus* 'pinkish brown, reddening inner stipe', *A.* 'brown speckled' and *A.* 'pink scales'. Three genera of the

Bankeraceae, *viz*. *Phellodon niger*, *Hydnellum* 'pink spines' and *Sarcodon* 'greening at base of stipe', were only found in 1898/1934 as were *Hebeloma* 'medium pink buff' and *Tricholoma* 'large pink'. No potential indicator species were found in 1934.

Discussion

Species richness, macrofungi on soil

The total number (330) of ectomycorrhizal fungi found (more than half the total number of macrofungal species found fruiting on soil) from 1ha of native wet sclerophyll *E. obliqua* dominated forest was generally higher than that from studies in Northern Hemisphere forest ecosystems (e.g. Termorshuizen 1991, 42 spp.; Nantel and Neumann 1992, 240 spp.; Visser 1995, 41 spp.; Senn-Irlet and Bieri 1999, 104 spp.; Smith *et al*. 2002, 215 spp.; Bonet *et al*. 2004, 144 spp.; Norvell and Exeter 2004, 309 spp.; Kranabetter *et al*. 2005, 128 spp.; Fernández-Toirán *et al*. 2006, 109 spp.). It was also higher than the number found in other Australian studies (Burns and Conran 1997, 26 spp.; Packham *et al*. 2002, 53 spp.; McMullan-Fisher 2008, 46 spp.; Newbound 2009, 37 spp.). As other researchers (e.g. Bills *et al*. 1986) have found, it is difficult to compare studies that have been carried out in different hemispheres, in different forests, on different soil types, at different altitudes, in stands of different ages, with a variety of microclimates. Studies vary in length of survey period, in sampling procedure, in area sampled and in operator expertise.

The data from the current study (Table 6.1) show that less than 50% of the total ectomycorrhizal species were collected from an area of 500m^2 in an *E. obliqua* wet sclerophyll forest but that ca. 80% were collected from an area of 1500m^2, the latter being a more informative data set from our forests. For comparison, Senn-Irlet and Bieri (1999) ascertain that their model based on 500m^2 was adequate for acquiring a reasonable amount of data on fungal diversity in a *Picea abies* forest in Switzerland.

A below-ground study in Tasmania (Tedersoo *et al*. 2008) reported a total of 123 putative ectomycorrhizal taxa from three plots totalling 3ha from an *Eucalyptus regnans*, *Pomaderris apetala* and *Nothofagus cunninghamii* forest. The current study from a *Eucalyptus obliqua, P. apetala, N. cunninghamii* forest, which produced almost three times the number of above-ground species was from an area equivalent

to one-third of their study area. The total ectomycorrhizal root associated fungal community was estimated by Tedersoo *et al*. (2008) to be between 210 (using estimator Chao2) and 247 (using estimator ACE) species, although those authors believe this to be an underestimate. This is approximately a third of what the current study was estimated to produce (710 from Chao2 and 696 from ACE, see Table 8.2) in above-ground ectomycorrhizal species. These discrepancies could suggest that: i) Tedersoo *et al*. (2008) had a very limited sampling procedure ii) that the molecular techniques were not picking up all the species, iii) that the taxonomy of the above-ground survey was erroneous, iv) that the above-ground fruit bodies were not forming mycorrhizae or v) the study sites were too different. However, Erland and Taylor (2002) consider it impossible to analyse more than a small fraction of the ectomycorrhizal community on root tips and many species must go unrecorded.

Some findings from the current study were consistent with those of other studies. For example, the percentage of ectomycorrhizal species fell within the estimate of 35-40% of total macrofungal species (Lange 1978, Watling 1995, Kendrick 2000) and there were a lot of species collected only once (as in Straatsma and Krisai-Greilhuber 2003, Bonet *et al*. 2004, Richard *et al*. 2004, Smith *et al*. 2002). The high number of singletons may be due to the limited survey period or else that these species are rare or do not fruit frequently.

A number of factors could be responsible for the very high number of ectomycorrhizal species found in the current study in a wet *E. obliqua* forest. These include the very intense sampling effort, the longer growing season in Tasmanian forests, the greater number of ectomycorrhizal hosts and number of living stems, the great ability of the genus *Eucalyptus* to form mycorrhizae (Ashton 1976a, Chilvers 2000, Bougher 1995), the older age of the stands and the fact that the study was conducted in a native forest, the only disturbance being that of wildfire. The sampling at fortnightly intervals did mean that there were some species probably not recorded but these would have been non-ectomycorrhizal, fragile, ephemeral genera, e.g. *Coprinellis, Parasola, Mycena, Mycenella,* whereas the fruit bodies of the ectomycorrhizal genera *Boletus, Cortinarius, Russula, Tricholoma, Inocybe,* and *Phellodon* are either relatively large and remain in a condition such that even after a

fortnight they can still be identified or are tough and fibrillose, resisting decay and attack by predators.

The ever-increasing species accumulation curves, irrespective of whether visits or subplots were used (Figures 6.4 and 6.5), are usual in this type of study. The authors of two works undertaken over 21 years (Straatsma *et al*. 2001, Tofts and Orton 1998) concluded that even after that length of time not all the species had been collected. Reflecting that "a complete list is an unattainable ideal", Tofts and Orton (1998) suggested that 25-30 years is a more appropriate time frame for meaningful results, although as Watling (1995) pointed out, "the effects of vegetation succession, acid precipitation and possibly global warming will be imposed on the recording".

Species identification of macrofungi on soil

The largest number of ectomycorrhizal species belonged to the family Cortinariaceae (258) across all four plots (Figure 6.2). Most of the ectomycorrhizal collections that could not be identified to species level belonged to the genus *Cortinarius* (as also found by Nantel and Neumann 1992, Visser 1995, Smith *et al*. 2002, Norvell and Exeter 2004, Trudell and Edmonds 2004, Bonet *et al*. 2004, Robinson and Tunsell 2007). The large number of ectomycorrhizal species of *Cortinarius* was confirmed by the below-ground results of Tedersoo *et al*. (2008), who found that the genus *Cortinarius*, colonizing 10.9% of root tips in their study, produced among the most abundant ectomycorrhizal taxa.

Assemblage composition and distribution of macrofungi on soil

The Old growth plot had the highest number of decomposer macrofungal species on soil (Figure 6.1). There was a layer of moss and well-rotted leaves on the floor of this plot (G. Gates pers. obs.), typical of an old wet eucalypt forest. Saprotrophic fungi are favoured by this resource, which maintains temperature and moisture (Straatsma *et al*. 2001, Fernández-Toirán *et al*. 2006) and is rich in organic matter. The 1934 plot had the lowest number of decomposer species (Figure 6.1), which may be related to the quantity and species of plant remains present in the soil resulting from the composition of the vascular plant community present. The steepness of the plot is not conducive to moisture retention (except in Tunna), an essential requirement (Ward *et al*. 1991) of dead plant material decomposition.

Although there was a pronounced plot influence on all macrofungi fruiting on soil (Figures 6.7 and 6.8), which was even more pronounced when ectomycorrhizal species were examined separately (Figures 6.9 and 6.10), the results cannot be explained due to differing inherent site characteristics. The results suggested that the mature forest had a different ectomycorrhizal community to that of the young forest (Figures 6.A1, 6.A2 and 6.A3). Between the mature plots (Old growth and 1898) the Old growth plot had the least number of misclassifications (2) for ectomycorrhizal species and visits and the 1898 plot the most (7). The Old growth plot as regards the vascular plant community could be considered the most stable and homogeneous plot as it had the lowest diversity of higher vascular plants. The 1898 plot was very variable in its vascular plant composition (Table 2.2), which could be reflected in the ectomycorrhizal assemblages. A below-ground study of ectomycorrhizal fungi in a birch and conifer plot regenerating from a 1870 fire and a birch and conifer plot regenerating from a 1916 fire (DeBellis *et al*. 2006) found that the ectomycorrhizal fungi showed different host preferences which resulted in plot differences. However, it is difficult to be definitive about the cause of the results in the current study, i.e. whether it was vegetation type, soil type, survey area, microclimates, slope or an interaction of these factors.

Phenology of ectomycorrhizal and decomposer macrofungi on soil

There was an optimum time of fruit body emergence for the majority of species (Table 6.A3), irrespective of the plot in which the species was found. Although it could be expected that good rainfall would maintain moisture in the soil and enhance fruit body production this does not appear to have happened. Fruit body manifestation was not unequivocally related to rainfall events, which agrees with Eveling *et al*. (1990). The numbers of species on soil in the 1898/1934 and Old growth plots were approximately double for the second season (May-June 2007). It is difficult to explain this discrepancy as collecting intensity was the same for both years, although yearly differences with no very clear relationship with weather variation are not uncommon (e.g. Lange 1984, Straatsma and Krisai-Greilhuber 2003).

Seasonal differences in fruiting patterns were better described using indigenous seasons (as with the wood-inhabiting macrofungi in Chapter 4). This is because the indigenous season Tunna contains the months of May-August, and May-July in both

2006 and 2007 produced the highest number of species (Figure 6.6). For all macrofungal assemblages on soil and for the ectomycorrhizal species separately, there was a greater response made by the 1934 plot to the use of indigenous seasons than any of the other three plots. The 1934 plot is steep (Table 2.1), without a dense understorey (Table 2.2). It is likely to be able to retain moisture only when temperatures became cooler and day length shorter, coinciding with the start of Tunna.

Old growth, compared to the other three plots, maintained continuous numbers of soil-inhabiting macrofungi throughout the year, which may reflect the greater moisture holding capacity of the plot. The all year round appearance of *Laccaria* spp. and *Lactarius eucalypti* could just reflect that these two species are the most commonly encountered in this forest type (Ratkowsky and Gates unpublished data, 1998-2008). They are also among the most common ectomycorrhizal species found by Tedersoo *et al*. (2008) in their below-ground study. A build up of vegetative mycelium below the soil is triggered by some factor into producing the sexual reproductive stage of the fungus' life cycle. The larger store of mycelium required for species with a large fruit body, e.g. the larger *Cortinarius*, *Lactarius* and *Russula* species (Bohus and Babos 1960, Wilkins and Harris 1946), would take a lot longer to build up compared to that of the smaller decomposer *Entoloma* and *Hygrocybe* species and the small ectomycorrhizal *Laccaria* spp. and *Lactarius eucalypti*. It is feasible that these species could be triggered to fruit more often, especially the more opportunistic decomposer species. The tough, leathery species, e.g. *Phellodon niger* and *P.* '*brown*', unlike the fleshy boletes, *Amanita, Cortinarius, Russula*, and *Tricholoma* species (Table 6.A3), which decay rapidly and are prone to being colonised by collembolan species (especially hypogasturids), mycetophilid flies and slugs (Keller and Snell 2002) are able to persist for many months. Taxa of the genus *Cortinarius* s.l. and other members of the family Cortinariaceae were responsible for the high numbers of species on soil in both Old growth and 1898/1934 (Figure 6.2). The time of appearance and disappearance of the majority of *Cortinarius* species may be related to physiological factors associated with tree growth (Bohus and Babos 1960). Other environmental factors (documented by Kües and Liu 2000), not measured in the present study, that affect primordia formation are: soil moisture, soil temperature, humidity, salinity, pH, light, CO_2 concentrations, the association with

micro-organisms and the presence of specific genes that contribute to initiation of fruit body formation.

Correlation and distribution of ectomycorrhizal species with vascular plant community and stand age

Although there was a correlation of the number of ectomycorrhizal macrofungal species with the 21 vascular plants present in the four plots (Figures 6.11) and the regression analysis (Figure 6.12) showed that the total number of ectomycorrhizal species on soil was correlated with the total number of living vascular plant species in each of the 100 subplots, there were site factors that could not be extracted from the analyses to give a conclusive explanation.

Although there was no correlation of ectomycorrhizal macrofungal species with the numbers of host trees, the 1898/1934 plot had the highest numbers of ectomycorrhizal fungal species and the highest number of ectomycorrhizal host trees (Table 2.2), including a high percentage of *Pomaderris apetala*, an understorey species known to form ectomycorrhizal associations (Ashton 1976a, Tedersoo *et al*. 2008). The 1898/1934 and 1934 plots (the two young plots) had almost the same numbers of the ectomycorrhizal host *E. obliqua* (39 and 40 respectively); however, *P. apetala* was absent from 1934 and in its place *Monotoca glauca* formed the sparse, scrubby understorey of the 1934 plot (Table 2.2). *Monotoca glauca* belongs to the family Epacridaceae, members of which form ericoidal mycorrhizae that do not produce conspicuous above-ground fruit bodies (Smith and Read 2008). The edaphic factors for the 1934 plot were different to the other three plots (Table 2.1). The more acidic soil of the 1934 plot favoured the growth of *Monotoca glauca* (Mark Neyland pers. comm.) and not *P. apetala* which is favoured by dolerite soils. Litter analysis by Ashton (1975) revealed that the litter of *P. aspera*, a species that is similar to *P. apetala* but which has leaves not densely covered in hairs on the lower surface, had a calcium content two to three times as great as that of *E. regnans*. The large number of living *P. apetala* stems in 1898/1934 is a potential source of large amounts of soft, readily decomposed leaves that are rich in calcium (probably in the form of calcium oxalate), if one extrapolates from the study by Ashton (1975). The presence of *Pomaderris* in the understorey can increase the soil pH by one whole unit (Ashton 1987). Ectomycorrhizal fungi thrive in the higher pH conditions caused by higher

calcium levels (Trocha *et al*. 2007). It has been shown that edaphic factors including pH influence the ectomycorrhizal community (McAfee and Fortin 1987, Tyler 1989, Rühling and Tyler 1990, Kranabetter 2005) and also indirectly determine plant community (Kernaghan *et al*. 2003), although ectomycorrhizal fungi correlate with vegetation independent of soil conditions (Nantel and Neumann 1992, Harrington 2003). It is possible that soil pH and/or humus pH, either directly or indirectly by influencing vegetation type and number of ectomycorrhizal hosts, could have contributed to the differences in ectomycorrhizal species in the 1934 and the 1898/1934 plots. The contribution of shrubby vascular plants to the study could not be evaluated as their ectomycorrhizal status was not known.

Although there were only four plots in this study, there is some support for the model of Dighton and Mason (1985) that proposes a decrease of species richness of ectomycorrhizal macrofungi in late successional stages of forest stands (see Figure 6.1). A decrease in the number of ectomycorrhizal species with increasing stand age was also found in above-ground studies (similar to the current study) by Termorshuizen (1991) and Smith *et al*. (2002) but not by Fernández-Toirán *et al*. (2006) and Visser (1995). Interestingly, in the current study, the largest number of ectomycorrhizal fungi, especially *Cortinarius* species (Figure 6.3) shared among the plots were those between 1934 and 1898/1934 (the younger forests) but were not found in Old growth or 1898 (the two mature plots), indicating that some successional processes could be occurring.

The uneven distributions of the above-ground fruit bodies and the positions of living trees (Figure 6.13(a-d)) may be explained by the distribution of below-ground ectomycorrhizae. In coniferous forests, the distribution of ectomycorrhizal species is usually clustered (Horton and Bruns 2001). Most species occur in less than 10% of soil cores taken and individual soil cores generally contain multiple species (Horton and Bruns 2001). The adjacent root tips are frequently colonised by different species (Horton and Bruns 2001). Also, ectomycorrhizal fungi can fruit many metres away from the host plant with genets of individual strains of ectomycorrhizal fungi being found 10-30m apart (Leake *et al*. 2002), so a patchy distribution above ground is to be expected (Koide *et al*. 2005). The trees surrounding the study area could have been associated with fruit bodies within the study area, further confounding the results.

Molecular techniques are currently used in other studies to determine the ectomycorrhizal species on the roots of the trees in an attempt to match them with the above-ground fruit bodies. This was not done in the current study, although a sample of each ectomycorrhizal species was frozen for DNA analysis, which would facilitate such a project in the future. In many studies there is rarely any correlation between the numbers and types of above-ground fruit body and below-ground ectomycorrhizal species as determined by molecular techniques, e.g. Peter *et al*. (2001a), Glen *et al*. (2008). This is because many species of ectomycorrhizal fungi do not form visible fruit bodies or fruiting is so sporadic over a survey period that they may never appear, or are missed when they do appear, due to the limits of the sampling procedure (Erland and Taylor 2002).

Potential ectomycorrhizal indicator species of stand age

Several taxa of the so-called 'early stage' genera of *Hebeloma, Laccaria* and *Inocybe* (Dighton and Mason 1985) were found only in the Old growth plot. The species of *Hebeloma* found in the Old growth plot (*Hebeloma* 'very large, spores 8x6') was not the same as the one found in the 1898/1934 plot (*Hebeloma* 'medium pink-buff, spores 8x5). The latter species is commonly found after disturbances, e.g. along road sides and in a 'clearfell, burn and sow' silvicultural coupe at time 26 months of regeneration after burning (Gates *et al*. 2005) and has probably remained a viable symbiont in the 1898/1934 plot for approximately 70 years, having colonised soon after the last fire in that plot. Marmeisse *et al*. (1998) suggest that members of the genus *Hebeloma* readily use nitrate and are early ectomycorrhizal pioneer occupants of disturbed habitats where it is most likely to encounter nitrogen in this form. Two species of the genus *Laccaria,* i.e. *Laccaria* sp. A and *Laccaria masonii,* were confined to the Old Growth plot. This is a consistent finding with that of other surveys in Tasmania (Ratkowsky and Gates unpublished data, 1998-2008). Fuhrer (2005) notes that these two species are usually found associated with *Nothofagus cunninghamii* in cool temperate rainforest. A molecular study could reveal whether these species are ectomycorrhizal with *N. cunninghamii* or old *E. obliqua*. Such differences at a species level in the genera *Hebeloma* and *Laccaria* suggest that the notion of 'early and late stage' ectomycorrhizal fungi is oversimplified.

In Tasmania, the ectomycorrhizal *Cuphocybe* sp. ('C162'), *Inocybe* 'very large, nodulose spores', *Laccaria* sp. A, *L. masonii* and *Dermoloma* 'brown-yellow' and *D.* 'grey' were only found in the 'Old growth' plot. The status of these species as potential indicator species or a suite of indicator species of this forest age and type is supported by their occurrence in other similar forest types (Packham et al., 2002; Ratkowsky and Gates unpublished data, 1998-2008). In this survey three ectomycorrhizal genera of the hydnoid family Bankeraceae, viz. *Phellodon niger*, *P.* 'brown', *Hydnellum* 'pink spines' and *Sarcodon* 'greening at base of stipe', were only found in the relatively young forest plots of 1934 and 1898/1934, but these species have also been observed in older forests in Tasmania (Ratkowsky and Gates 2005, Ratkowsky and Gates unpublished data, 1998-2008).

It has been suggested that the domination of the ectomycorrhizal communities by certain genera is related to the mycelial morphology. Agerer (2001) has proposed a classification whereby the genera such as *Cortinarius, Tricholoma,* and *Hydnellum* contain many species that produce rhizomorphs, cords, or mats. These structures could be adaptive in habitats that experience strong seasonal drought (Unestam 1991, Unestam and Sun 1995). Trudell and Edmonds (2004) concluded that moisture differences were responsible for the differences in the ectomycorrhizal communities (which supported the classification of Agerer 2001) at two different sites of similar vegetation type. Members of the above suite of genera were common in the ectomycorrhizal community of the 1934 and 1898/1934 plot with the genus *Cortinarius* being very well represented. Both these plots were steeper than the two older plots, implying greater water run-off and drier conditions. These moisture differences among plots suggest yet another ecological process that could be interacting with those already discussed.

Conclusions

The present study:

- found that the native wet *E. obliqua* forests of southern Tasmania support a large and diverse community of macrofungi fruiting on soil, many species of which are previously undiscovered and undescribed.

- identified that the majority of these new species belong to the genus *Cortinarius*, which is known to form many ectomycorrhizal associations with eucalypts. The biological properties and roles of these fungi in the forest ecosystem are unique but unfortunately largely unknown (Bougher 1995). There are great benefits to forestry in ecological and commercial terms in having these species discovered and characterised.
- found that each plot supported unique species assemblages fruiting on soil; the plot differences were even more pronounced for the ectomycorrhizal fungal communities.
- suggested some evidence of ectomycorrhizal succession, as the most number of shared species was between the younger plots.
- determined that neither rainfall nor temperature could separately explain conclusively the variation in fruiting body emergence of macrofungi fruiting on soil.
- revealed that most species fruiting on soil emerged in the indigenous season of Tunna (May-August) in all four plots.
- provided valuable information that contributes to benchmark data on the diversity and frequency of macrofungi fruiting on soil with special reference to ectomycorrhizal species in native *E. obliqua* forest stands of different ages since wildfire in southern Tasmania.
- showed that there was stability of the ectomycorrhizal communities across time and space within a plot.
- was unable to correlate ectomycorrhizal species richness with the abundance of the three main ectomycorrhizal host species (viz. *E. obliqua*, *N. cunninghamii*, *P. apetala*), although there was a highly significant correlation with the total number of living stems at the subplot level. However, because individual fruit bodies of ectomycorrhizal species were not traced to their host species, the above finding cannot be explained.
- highlights that works of longer duration and more intensive sampling are needed to obtain data regarding ectomycorrhizal fungal communities, with more attention to specific variables such as microclimate, soil moisture, soil type, soil pH and vegetation types.

- hypothesises that more ectomycorrhizal species would have been found if attention could have been focussed on hypogean fungi and ectomycorrhizal root tips.
- reveals that more ecological work on ectomycorrhizal fungi is needed in native eucalypt forests in earlier stages of regeneration since wildfire, silviculture treatments and in plantations of different ages.
- proposes that studies involving the hypogean fungal communities, mapping above-ground fruit bodies, and using molecular techniques on below-ground mycorrhizae would be ideal projects to follow up the results obtained from this study.